ALLOSTERIC MODULATORS OF FGF/FGFR SIGNALLING AS INNOVATIVE TOOLS AGAINST CANCER AND OTHER FGFR DRIVEN PATHOLOGIES

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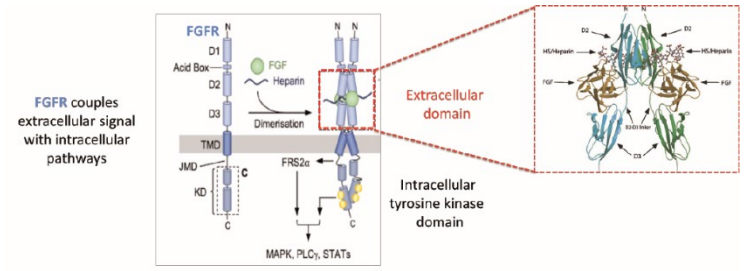
Fibroblast growth factor (FGF2)/fibroblast growth factor receptor (FGFR) signalling is involved in several pathologies, including cancer development, metastasis formation and resistance to therapy, vascular diseases, and viral infections. The development of small molecules, acting extracellularly to target FGF2/FGFR interactions, has the advantage of limiting the adverse effects associated with current intracellular FGFR inhibitors.

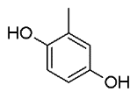


We identified a few leads, either targeting FGF2 [1, 2] or FGFR-D2 domain [3], able to destabilize the FGF2/FGFR complex and inhibiting the subsequent signalling cascade triggered by FGFR TK domain phosphorylation.

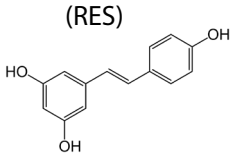
Unrestrained docking

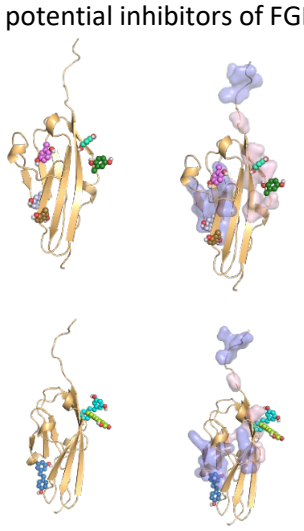
Unrestrained docking approaches (HADDOCK) were employed to screen, among a small library of natural compounds proposed to affect FGF2/FGFR signalling pathway,the potential inhibitors of FGF2/FGFR interaction.

Toluquinol (TOL)



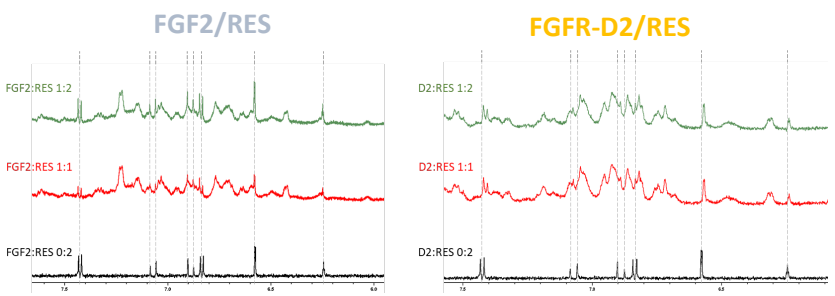
Resveratrol (RES)



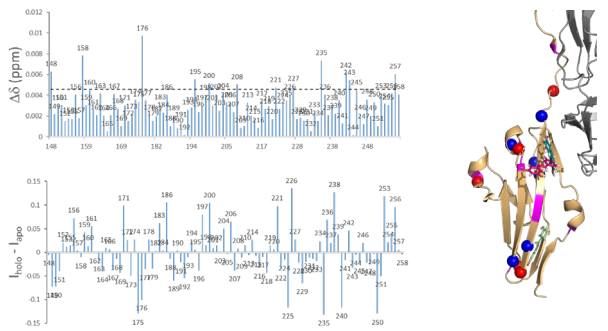


Both RES and TOL bind to regions close to the identified FGFR-D2 key regions

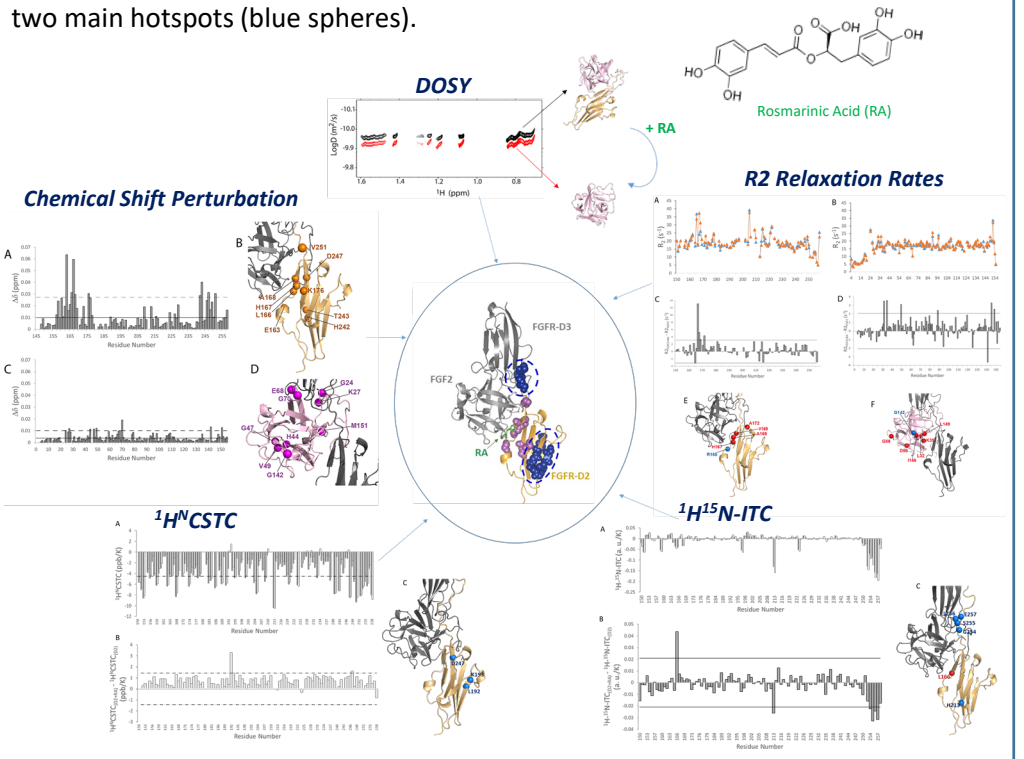
RES preferentially binds to the FGFR-D2 domain



No significant perturbation was observed upon RES addition to FGF2
Both RES and FGFR-D2 are perturbed indicating the presence of specific interaction in the micromolar range



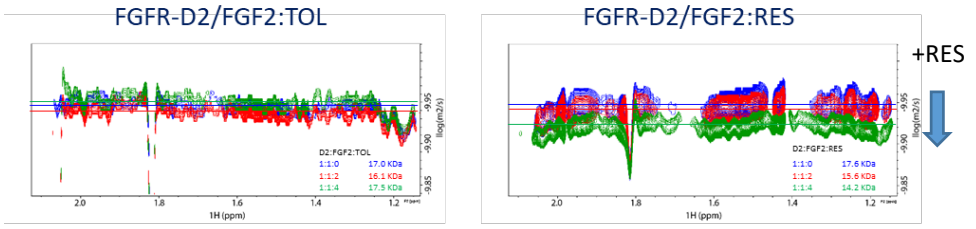
We recently demonstrated [3] that the natural compound RA is able to specifically bind to FGFR-D2 domain, hindering the FGF2/FGFR-D2 interface (pink spheres).
RA binding stabilises the dissociated form (DOSY). NMR chemical-shift perturbation analysis, R2 and temperature coefficient data demonstrate that allosteric effects, induced by RA binding to FGFR-D2, are gathered at the level of two main hotspots (blue spheres).

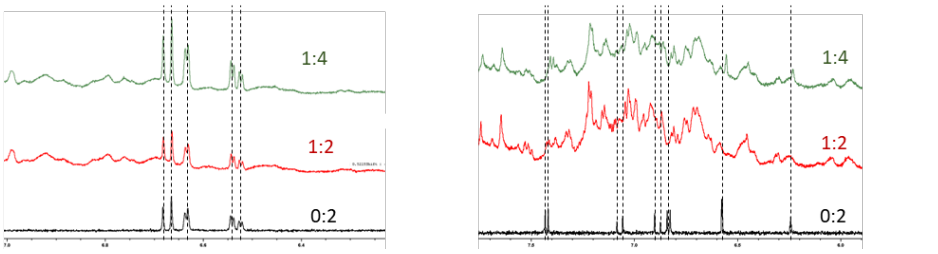


Cellular assays demonstrate that RA inhibits FGF2-induced endothelial cells proliferation and receptor activation.

RES dissociates the complex in a dose dependent manner

DOSY experiments were used to evaluate natural compounds efficacy in destabilizing the complex.





TOL does not induce complex dissociation. Resonances don't show significant perturbations in the presence of FGFR-D2/FGF2 complex

RES resonances shift and broaden when added to FGFR-D2/FGF2 complex, pointing to its binding to the complex or to one of the complex domains

Work in progress

- NMR characterization of RES induced allosteric perturbations on FGFR2-D2 domain to verify whether a common mechanism of action, involving the same hot spot regions identified for RA, is at work.
- Cellular studies to test RES and TOL ability to interfere with FGF2-induced endothelial cell proliferation and FGFR activation

References
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[3] K. Pagano, L. Carminati, S. Tomaselli, H. Molinari, G. Taraboletti, L. Ragona *ChemBioChem*, **1**, 160-169 (2021)

Acknowledgements
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